Attorney Docket No.: 4394.214-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Svendsen et al. Confirmation No: 3011

Serial No.: 09/325,603 Group Art Unit: 1652

Filed: June 3, 1999 Examiner: E. Slobodyansky

For: α-Amylase Variants

Pending Claims

81. A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca2+ -dependency and specific activity, wherein said parent alpha-amylase has a sequence of at least 70% homology to the sequence of SEQ ID No: 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.3) using default values for GAP penalties, said method comprising

(a) generating a three dimensional model of a parent alpha-amylase structure, utilizing data from Appendix 1 and a computer programmed for generating said model from said data;

(b) identifying in said three-dimensional parent alpha-amylase structure generated in step (a) at least one amino acid residue or at least one structural part; wherein an alteration in said at least one amino acid residue or said at least one structural part is predicted to result in said altered property;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue or at least one structural part identified instep (b); and

(d) expressing the modified nucleic acid of step (c) in a host cell to produce said variant alpha amylase.

83. The method according to claim 81, wherein said three-dimensional alpha amylase structure has an A domain, a B domain and a C domain, wherein said A domain has an amino acid sequence corresponding to residues 1-103 and 206-395 of SEQ ID NO: 2; said B domain has an

amino acid sequ nce corresponding to residues 104-205 of SEQ ID NO:2 and said C domain has an amino acid sequence corresponding to residues 396-483 of SEQ ID NO:2.

87. A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase wherein said altered property is increased calcium binding affinity, said method comprising

(a) generating a model of a three dimensional structure of a parent alpha-amylase, using the coordinates of the three dimensional structure of SEQ ID NO:13 depicted in Appendix 1 and a computer programmed for generating a model structure, said parent alpha-amylase having at least 70% homology to SEQ ID NO:13,

(b) utilizing said three dimensional structure generated in step (a) and modeling methods to identify in said parent alpha-amylase structure at least one amino acid residue or structural part within 10Å from a calcium binding site;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue identified in step (b); and

(d) expressing the modified nucleic acid in a host cell to produce said variant alpha-amylase.

88. A method according to claim 87, wherein the variant has a decreased calcium ion dependency of enzymatic activity or stability.

89. A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase wherein said altered property is selected from the group consisting of pH optimum and the enzymatic activity at a given pH, said method comprising

(a) generating a model of a three dimensional structure of a parent alpha-amylase, using the coordinates of the three-dimensional structure of SEQ ID NO:13 depicted in Appendix 1 and a computer programmed for generating a model structure, said parent alpha-amylase having at least 70% homology to SEQ ID NO:13;

(b) utilizing said three dimensional structure generated in step (a) and modeling methods to identify in said parent alpha-amylase structure at least one amino acid residue or structural part within 15Å from an active site residue;

(c) modifying the sequ nce of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue identified in step (b); and

(d) expressing the modified nucleic acid in a host cell to produce said variant alpha-amylase.

90. The method according to claim 89, wherein the variant has an altered pH optimum relative to the parent.

91. A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase wherein said altered property is selected from the group consisting of substrate specificity, substrate binding and substrate cleavage pattern said method comprising

(a) generating a model of a three dimensional structure of a parent alpha-amylase, using the coordinates of the three-dimensional structure of SEQ ID NO:13 depicted in Appendix 1 and a computer programmed for generating a model structure said parent alpha-amylase having at least 70% homology to SEQ ID NO:13;

(b) utilizing said three dimensional structure generated in step (a) and modeling methods to identify in said parent alpha-amylase structure the substrate binding site;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids of the substrate binding site identified in step (b); and (d)

expressing the modified nucleic acid in a host cell to produce said variant alpha amylase.

93. A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca2+ -dependency and specific activity, wherein said parent alpha-amylase has an amino acid sequence having at least 70% homology to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO:6, when homology is determined by the GAP program (Genetic Computer Group, V rsion 7.3) using default values for GAP penalties, said method comprising:

(a) identifying in a model of a three-dimensional structure of said parent alpha-amylase at least one amino acid residue or at least one structural part; wherein an alteration in said at least one amino acid residue or said at least one structural part is predicted to result in said altered property, wherein said model was generated using a computer programmed for generating said model and wherein said model displays the coordinates for the three dimensional structure for SEQ ID NO:13 shown in Appendix 1 adapted to said parent alpha-amylase;

(b) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue or said at least one structural part identified in step (a); and

(c) expressing said modified nucleic acid of step (b) in a host cell to produce the variant alpha-amylase.